Report

Current Biology

Prosocial Choice in Rats Depends on Food-Seeking Behavior Displayed by Recipients

Highlights

- We developed an automated two-choice task to study prosocial behavior in rats
- Rats showed a high proportion of prosocial choices in the absence of self-benefit
- We were able to manipulate our task to disentangle mechanisms of prosocial choice
- Recipients' food-seeking behavior is necessary to drive prosocial choice

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In Brief

Márquez et al. show that rats behave prosocially in a food-foraging task. By manipulating the behavior of, and reward to, recipients of help, they disentangled the proximate mechanisms of prosocial choice, showing that rats are sensitive to food-seeking behavior and reward delivered to conspecifics.

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Prosocial Choice in Rats Depends on Food-Seeking Behavior Displayed by Recipients

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SUMMARY

Animals often are prosocial, displaying behaviors that result in a benefit to one another [1–15] even in the absence of self-benefit [16–21] (but see [22–25]). Several factors have been proposed to modulate these behaviors, namely familiarity [6, 13, 18, 20] or display of seeking behavior [16, 21]. Rats have been recently shown to be prosocial under distress [17, 18] (but see [26–29]); however, what drives prosociality in these animals remains unclear. To address this issue, we developed a two-choice task in which prosocial behavior did not yield a benefit or a cost to the focal rat. We used a double T-maze in which only the focal rat controlled access to the food-baited arms of its own and the recipient rat's maze. In this task, the focal rat could choose between one side of the maze, which yielded food only to itself (selfish choice), and the opposite side, which yielded food to itself and the recipient rat (prosocial choice). Rats showed a high proportion of prosocial choices. By manipulating reward delivery to the recipient and its ability to display a preference for the baited arm, we found that the display of food-seeking behavior leading to reward was necessary to drive prosocial choices. In addition, we found that there was more social investigation between rats in selfish trials than in prosocial trials, which may have influenced the focals' choices. This study shows that rats provide access to food to others in the absence of added direct self-benefit, bringing new insights into the factors that drive prosociality.

RESULTS AND DISCUSSION

Prosocial Choice

It has been proposed that the neurobiological systems at the basis of social behaviors such as empathy and helping conspecifics may be shared across mammalian species [30]. Still, the scarcity of evidence for prosocial behavior in animal species amenable to mechanistic studies and the difficulty in establishing behavioral paradigms that allow the dissection of the proximal factors promoting or constraining prosocial behavior have hindered the search for the mechanisms of prosociality. Thus, the development of new paradigms to study social behaviors, such as prosocial choice, under highly controlled environments and using laboratory animal models, will greatly benefit the search for generic mechanisms underlying prosociality. Rats, a social species widely used in neuroscience, have been shown to cooperate in tasks testing for coordination and direct and generalized reciprocity [2, 8, 9, 11, 31], and to possess the cognitive capacity to engage in cooperation in the context of social dilemma games [32]. More recently, rats have been shown to release a conspecific from a restrainer, a prosocial act that may not involve a benefit to the focal [17, 18]. Nonetheless, what drives the release of the restrained rat remains unclear [26–29]. In addition, to our knowledge, there are no published attempts at probing this form of prosocial behavior in the absence of stress. Tasks that use food rewards allow for a better control over the behavior of subjects and the outcomes of particular action choices, permitting the disentangling of factors that drive prosocial behavior. Therefore, we set out to develop a reward-based task to study the mechanisms of prosocial behavior without self-benefit in Sprague-Dawley rats. The task we developed was inspired in classical two-choice tasks used to study prosociality in other species, such as a primates and corvids [19, 33, 34].

To this end, we developed a fully automated double T-maze in order to minimize interference by the experimenter while at the same time allowing for a precise control and detailed monitoring of the behavior of the interacting individuals. In each maze, a center arm gave access to two food-baited arms gated by automated doors. Pairs of non-food-deprived cage-mate rats were tested in our double T-maze (one per rat). For each pair, one rat was assigned to be the focal (the decision maker in our task) and the other the recipient (whose access to the rewarded arms depended on the focal). The focal could choose between the side that provided food only to itself (the focal received one food pellet and the recipient none; selfish choice) and the opposite side, which provided food to itself and the recipient rat (both focal and recipient received one food pellet; prosocial choice). Thus, prosocial choice did not imply an added benefit or a cost to the focal rat. Because in our task there was no role reversal (the focal never took the place of the recipient and vice versa), there was no room for reciprocal cooperation to emerge.

Since it has been shown in chimpanzees that behavioral displays of intention are required for the focal to provide help [16, 21], in our task recipient rats were trained to display food-seeking behavior. This corresponded to poking a nose port that controlled the automated doors giving access to the side arms of each maze. The nose ports were placed above each door, such that when a rat would poke the nose port, the door underneath would open (see Figures 1A–1C and S1).

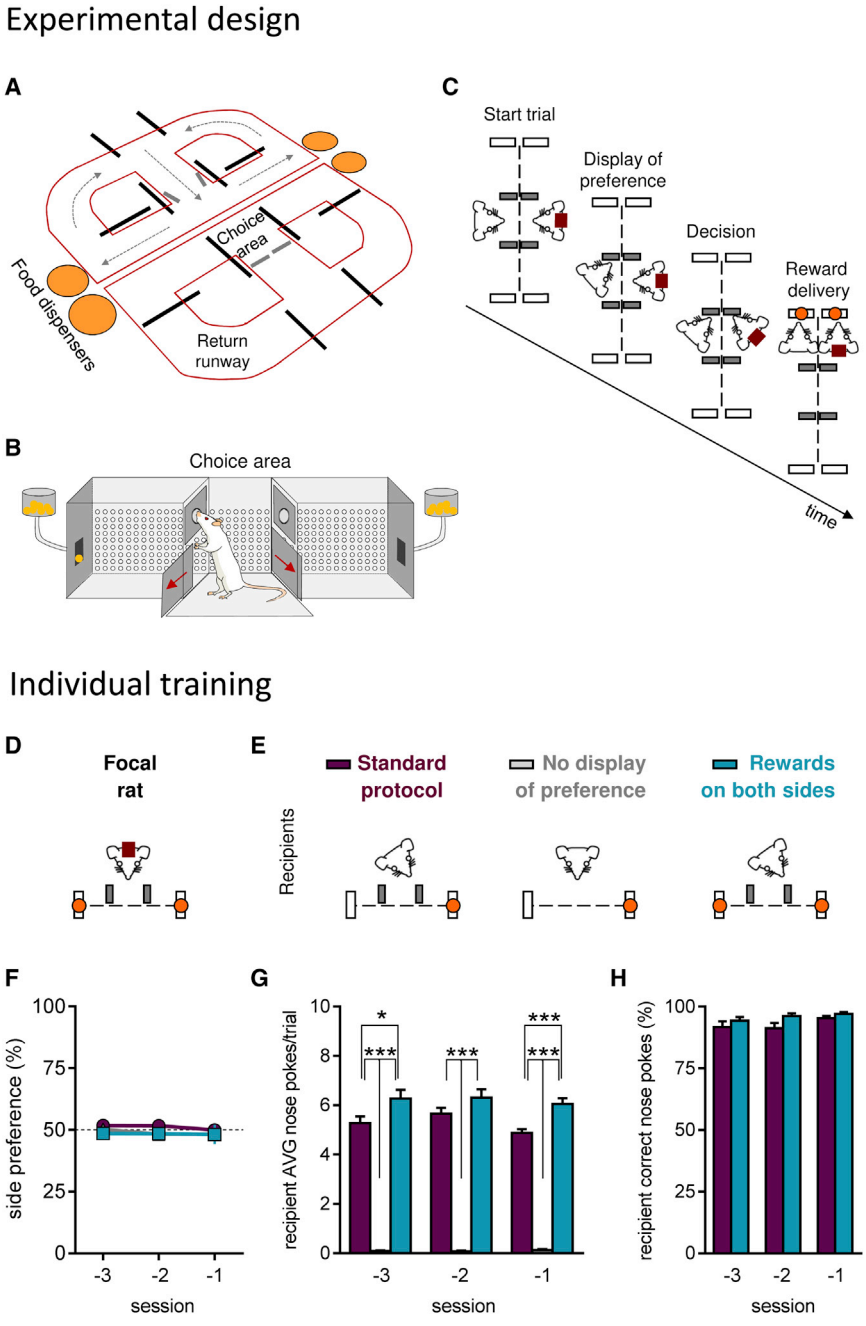

Figure 1. Two-Choice Task for the Study of Prosocial Behavior in Rats: Apparatus, Experimental Design, and Individual Training

The apparatus and experimental design of the prosocial choice task (A–C) and the individual training of rats prior to testing (D–H) are presented.

(A) Schematic view of the double T-maze. Each T-maze (one per rat), consisted of a center arm that gave access to two arms gated by automated doors (black lines), at the end of which food was delivered. Arrows in the upper maze represent the flow of movement of rats in the maze. Access to the choice area was prevented by automated doors placed in the central arm (gray lines).

(B) The doors in the choice area were controlled by nose ports placed above them, such that when a rat would poke the nose port the door underneath would open. See also Figure S1.

(C) Schematic view of a trial. A trial started when both rats were in the central arm. Once the recipient rat started displaying food-seeking behavior (nose pokes on preferred side), the nose ports of the focal rat became active and the focal rat could choose to nose poke on either side of its own maze. A single nose poke by the focal rat opened the door underneath the port and the door on that same side of the recipient's maze. Then, both animals entered the lateral arm and were rewarded according to experimental protocol. In the schema, the focal rat is represented with a red square, reward as orange circles, nose ports that control the opening of the doors as gray rectangles, and side preference as the angled head of the recipient toward the nose port of one side.

(D and E) The different individual training procedures for focal (D) and recipient (E) rats in "standard" and control protocols are schematized using the same symbols as in (C).

(F) Focal rats showed no side preference at the end of training, where side preference reports the percentage of choices to the arm that corresponded to the prosocial side during testing (since sides were counterbalanced across rats, for some animals side preference was the number of choices for the left side and for others the right side) (one-sample t test against chance for each experimental protocol independently, "standard" protocol: $t_{(14)} = 0.872$, $p' = 0.398$ for session −3; $t_{(14)} = 0.971$, $p' = 0.348$ for session −2; $t_{(14)} = -0.040$, $p' = 0.969$ for session −1; "no display of preference" protocol: $t_{(10)} = 0.105$, $p' = 0.918$ for session −3; $t_{(10)} = -1.082$, $p' = 0.304$ for session −2; $t_{(10)} = -1.037$, $p' = 0.324$ for session −1; "reward on both sides" protocol: $t_{(10)} = -0.763$, $p' = 0.463$ for session −3; $t_{(10)} = -0.713$, $p' = 0.491$ for session −2; $t_{(10)} = -0.471$, $p' = 0.647$ for session −1).

(G) The number of nose pokes per trial displayed by recipient rats differed across the three protocols. As expected, the number of nose pokes in the "no display of preference" condition was negligible. In addition, recipients of the "reward on both sides" protocol had a higher rate of nose pokes when compared to the "standard" condition (one-way ANOVA comparing experimental protocols in each training session, session −3: $F_{(2,34)} = 144.313$, $p < 0.00001$, further Fisher's least significant difference (LSD) post hoc tests revealed significant differences between "standard" and "reward on both sides" protocols [$p' = 0.011$] and between these two protocols and "no display of preference" protocol [$p' < 0.00001$]; session −2: $F_{(2,34)} = 192.864$, $p < 0.00001$, further LSD post hoc revealed a marginally significant difference between "standard" and "reward on both sides"' protocols [$p' = 0.058$] and a significant difference between these two protocols and "no display of preference" protocol [$p' < 0.00001$]; session −1: $F_{(2,34)} = 349.925$, $p < 0.00001$, further LSD post hoc revealed significant differences between "standard" and "reward on both sides" protocols [$p' < 0.0001$] and between these two protocols and "no display of preference" protocol [$p' < 0.00001$]).

(H) Recipients nose poked almost exclusively in the active port and in a similar manner in both conditions where animals were trained to display food-seeking behavior (independent sample t test for each training session: $t_{(24)} = 0.080$, $p = 0.937$ for session −3; $t_{(24)} = -1.057$, $p = 0.301$ for session −2; $t_{(24)} = -1.260$, $p = 0.220$ for session −1). Mean ± SEM are shown. *$p' < 0.05$, **$p' \leq 0.005$, ***$p' \leq 0.001$.

Corrected p values (p') after sequential Bonferroni correction for multiple comparisons are reported; three comparisons were performed for each protocol in (F) and each testing session in (G).

Before testing, both rats were trained individually to poke in the nose ports in order to open the doors of the food-baited arms, retrieve the food reward, and run around the maze to the choice area, initiating a new trial. Focals were trained until no side bias was observed. Training of recipients depended on experimental protocol (Figures 1D–1H and Supplemental Experimental Procedures). Once individual training was complete, rats were tested for prosocial behavior in the double T-maze for four daily test sessions of 40 min, during which rats completed an average of 32 trials.

During testing for prosocial behavior, both rats had access to the nose ports of their corresponding mazes; however, only the ports of the focal were active, and these controlled the doors of both mazes. In this manner, the recipient rat displayed food-seeking behavior (poking the deactivated port) while the focal controlled the recipient's access to the food-baited arms. It has previously been shown that under some circumstances rats seek proximity with conspecifics [24, 31, 35]. To avoid a contribution of the preference for being together to prosocial choice, in both prosocial and selfish choices, we ensured that focal and recipient rats always went to the same side of the maze, independently of whether recipients received reward or not. Importantly, rats could see, smell, hear, and partially touch each other through a transparent perforated wall that separated the two mazes. Hence, they could interact at the decision and reward areas. Finally, rats were synchronized such that the focal could always observe the recipient's food-seeking behavior at the choice point and its consumption of the food pellet at the reward area. To this end, the ports of the focal rat were only active once the recipient started poking its nose port, and the food pellet to the recipient would only be delivered once the focal rat had entered the reward area (for details, see Figure S1 and the Supplemental Experimental Procedures).

We first asked whether a rat (the focal) provides access to food to another rat (the recipient) in the absence of direct self-benefit. We found that rats quickly acquired a preference for the prosocial option, providing the recipient with access to the food-baited arm (Figure 2A, left panel, and Figure 2B, purple line). The proportion of prosocial choices was higher than chance in all testing sessions, already being significant on the first one (one-sample t test against chance, defined as 50% choice: $t_{(14)} = 3.14$, $p' = 0.014$ for session 1; $t_{(14)} = 2.64$, $p' = 0.019$ for session 2; $t_{(14)} = 4.91$, $p' = 0.001$ for session 3; $t_{(14)} = 4.31$, $p' = 0.002$ for session 4). This preference became higher and more reliable over the course of the daily test sessions (repeated-measures ANOVA with "test session" as within-subject factor: $F_{(3,42)} = 3.392$, $p = 0.027$; tests of within-subject contrasts revealed a linear effect: $F_{(1,14)} = 11.41$, $p = 0.005$). However, even though focals' choices at the end of individual training (baseline) were not different from chance (one-sample t test against chance: $t_{(14)} = 0.67$, $p' = 0.513$), it is still possible that small biases for a particular side of the maze could account for the observed preference for the prosocial side. To test this, we compared the proportion of prosocial choices during testing to the proportion of choices for the same side during individual training. A significant difference between baseline and the first prosocial test session was found, indicating that minor individual biases for a particular side of the maze does not account for the preference for the prosocial side observed during early testing (paired-sample t test, baseline against the first prosocial test session: $t_{(14)} = -2.90$, $p = 0.023$).

The preference for the prosocial side, apparent already in the first session, could result from a bias toward the prosocial side at the very beginning of testing possibly as a result of local enhancement or behavior copying. Thus, we further analyzed the dynamics of the preference for the prosocial choice by quantifying the proportion of prosocial choices for the first, middle, and last third of each session, focusing on the first session. We found that animals started at chance and a marginally significant bias for the prosocial side appeared toward the end of the first session (one-sample t test against chance: $t_{(14)} = 0.95$, $p' = 0.358$ for the first third of session 1; $t_{(14)} = 2.12$, $p' = 0.104$ for the middle third of session 1; $t_{(14)} = 2.66$, $p' = 0.056$ for the last third of session 1; see Figures 2C and S2). This result shows that focal rats rapidly, but gradually, acquired a preference for the prosocial side, possibly through learning of the contingency between their choice and the outcome to the recipient. Although most rats showed a reliable preference for the prosocial side (see Figure 2D, purple dots), the strength of this preference varied substantially ranging from 60% to 89% (for each rat, an exact test was performed in which the null hypothesis was that rats chose at chance, revealing that ten out of 15 had a significant preference for the prosocial side, one showed a preference for the selfish side, and four remained at chance).

Multiple factors could explain the observed preference for the prosocial choice that may or may not correspond to some form of other-regarding behavior. For example, reward delivery to the recipient could have triggered a reward signal in the focal's brain (vicarious reward), reinforcing prosocial choice. In addition, as observed in prior studies [16, 21], focals could be reacting to the display of food-seeking behavior of the recipients.

Sensitivity to the Display of Food-Seeking Behavior

To test the role of food-seeking behavior on prosocial choice we performed, in parallel, a second experiment with a different set of animals, in which recipient rats were not allowed to display a preference for the rewarded side before the focal made its choice. Recipient rats were held away from the choice area by a door in the center arm until the focal made its choice, being thus prevented from poking the nose ports or showing other forms of preference for the baited side (training rats to poke the nose port greatly decreased other behaviors at the choice area; still, sometimes we could observe behaviors such as gnawing on the door of the rewarded arm; see Movie S1). Therefore, in this experiment, the focal rat had to choose without the display of preference by the recipient rat. After the focal's decision, the central door that held the recipient rat opened, and the experiment proceeded in the same manner as in the "standard" condition. As in the "standard" protocol, recipient rats received food on only one side of the maze. Thus, access to food depended on the focal's choices ("no display of preference" condition; Figure 2A, middle panel). Figure 2B (gray line) shows that unlike in the first experiment, focal rats stayed at chance levels throughout all test sessions, showing no preference for the prosocial side (one-sample t test against chance: $t_{(10)} = -1.31$, $p' = 0.219$ for session 1; $t_{(10)} = -0.68$, $p' = 0.509$ for session 2; $t_{(10)} = -0.10$, $p' = 0.921$

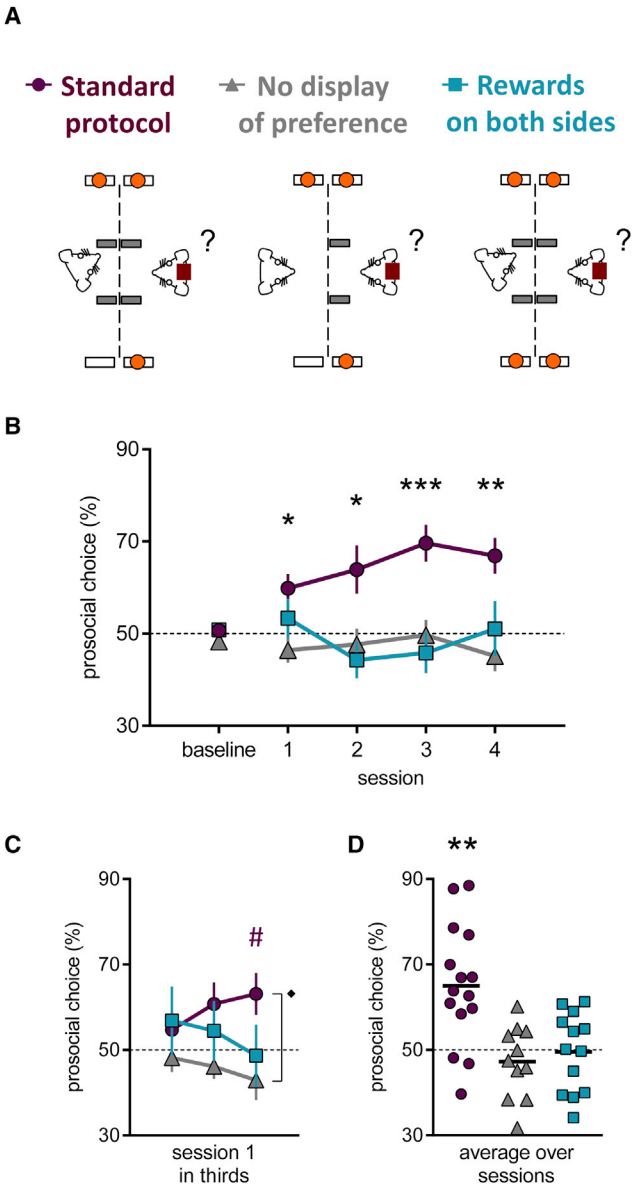

Figure 2. Prosocial Behavior in the Two-Choice Task

(A) Schematic views of the different protocols used to disentangle factors driving prosocial behavior are depicted using the same scheme as in Figure 1C. In all protocols, focal rats were always rewarded with one food-pellet, independently of choice, ensuring equal cost and benefit for both arms. In the "standard" protocol (left), recipients displayed food-seeking behavior by nose poking on the side where they would be rewarded. A prosocial choice (upper arm) resulted in reward to both animals, and a selfish choice (bottom arm) resulted in reward only to the focal animal. In the "no display of preference" protocol (middle), the recipient was prevented from accessing the choice area before the focal made its choice. Rewards were delivered as in the "standard" protocol—i.e., prosocial choice (upper arm), one food-pellet each, and selfish choice (bottom arm), one food-pellet to the focal rat only. Finally, in the "reward on both sides" protocol (right), the recipient still displayed food-seeking behavior toward one arm (in this case, the upper arm), but now one food-pellet was delivered to both animals on either side of the maze.

(B) Line graph shows that focal rats from the "standard" protocol (n = 15), but not the other two protocols (n = 11), quickly acquired a preference for the prosocial option, providing the recipient with access to the food-baited arm. Mean ± SEM of the percentage prosocial choices is shown for each experimental condition. Baseline and four test sessions are shown. Baseline corresponds to the percentage of choices for the arm that would later correspond to the prosocial side during testing, averaged across the last 2 days of individual training. One-sample t tests were used to compare proportion of prosocial choice in each test session within experimental groups against chance, and p values were adjusted after sequential Bonferroni correction. Repeated-measures ANOVA with "session" as a within-subject factor and "protocol" as a between-subject factor was performed to study differences between the different experimental protocols (see the main text).

(C) To study the emergence of prosocial choices within the first testing session, we divided performance in thirds. The percentage of prosocial choices for each third in each experimental protocol was then compared to chance (50%). No significant differences against chance were observed in the initial phase of testing, but a marginally significant preference toward prosocial choice emerged at the end of the session only in the "standard" protocol (see Figure S2 for further details). $^{\#}p' < 0.1$, deviation from chance; $^{\blacklozenge}p < 0.05$, difference between protocols.

(D) Individual values of prosocial choices averaged across sessions are plotted for each experimental condition. One-way ANOVA revealed differences between protocols, the "standard" protocol being significantly different from the other two conditions.

$^{*}p' < 0.05$, $^{**}p' \leq 0.005$, $^{***}p' \leq 0.001$. Corrected p values (p′) after sequential Bonferroni correction for multiple comparisons are reported; four comparisons we performed in (B) and three in (C). See also Figure S2 and Movie S1.

for session 3; $t_{(10)} = -1.46$, $p' = 0.176$ for session 4). This result shows that the recipient's food-seeking behavior was necessary to drive prosocial choice, which could be explained by a contribution of local enhancement, as observed in corvids [19], behavior copying, or a response to nose poking as a cue that may involve some form of mirror representation of the other's goals [21]. In addition, it shows that the cues related to reward delivery to the recipient rat, such as pellet odor, the sound of the pellet dropping, or chewing by the recipient, were not sufficient to drive prosocial choices. Finally, it suggest that although Norway rats in semi-natural conditions are tolerant to the presence of other rats at feeding sites [36, 37], feeding together does not by itself explain prosocial choices observed in the "standard" condition (first experiment). Analysis of deviation from chance for the choices of each focal rat showed that most rats remained at chance (seven out of 11), three displayed a preference for the selfish side, and only one rat showed a preference for the prosocial side. If eating together at least partially drove the focal rats' choices, a slight bias toward the prosocial side from some of the focal rats would be expected (a possibility even in the absence of a significant preference at the group level). This was, however, not the case.

Sensitivity to Reward Delivery

Our results show that focal rats are sensitive to the food-seeking behavior of the recipients, which could be the sole driver of prosocial choice. Alternatively, behavioral displays by recipients could be necessary, but not sufficient, to drive the preference for the prosocial side observed in the "standard" protocol. Focal rats may be sensitive to the reward delivered to the recipient rat, which together with the food-seeking behavior could drive prosocial choices. Indeed, it has been recently shown that upon the observation of a conspecific eating food, there is an initial increase in dopamine in the nucleus accumbens [38]. To test whether prosocial choices were driven solely by recipients'

food-seeking behavior and whether local enhancement or behavior copying could account for the focals' decisions, we ran a third experiment in another set of animals in which recipient rats still poked the nose port on one side only but received food pellets on both sides of the maze (see Figure 2A, right panel). In this manner, food-seeking behavior (poking the nose port on one side) was dissociated from the delivery of food (on both sides). If behavior copying or local enhancement explains the preference for the prosocial side observed in the "standard" protocol, then in this experiment focal rats should also show a preference for the side at which recipient rats poked. In contrast, if food delivery to the recipient rat also contributed to prosocial choice, in this experiment focal rats should remain at chance. In order to dissociate reward delivery from the side of nose poking, we changed the last 3 days of individual training of recipient rats, ensuring that they learned that poking the port on one side only was necessary to receive reward on either side of the maze (see Figure 1 and the Supplemental Experimental Procedures). In this third experiment, the behavior of the focal rat always led to reward to the recipient. Still, for simplicity and comparison with the previous experiments, we called the side of recipient nose poking the prosocial side. We found that focal rats stayed at chance level (Figure 2B, blue line), showing no preference for the side where the recipient was poking, conventionally called the prosocial side (one-sample t test against chance: $t_{(10)} = 0.79$, $p' = 0.449$ for session 1; $t_{(10)} = -1.44$, $p' = 0.181$ for session 2; $t_{(10)} = -0.94$, $p' = 0.368$ for session 3; $t_{(10)} = 0.17$, $p' = 0.871$ for session 4). This result suggests that rats were sensitive to the reward delivered to their cage-mates and that behavioral copying and local enhancement were not sufficient to sustain prosocial choices.

Comparison across Experimental Conditions

Next, we compared directly the choices of focal rats across the three experimental protocols over the course of the four testing days. To this end, we performed a repeated-measures ANOVA with "protocol" as a between-subjects factor and "test session" as a within-subjects factor. This analysis revealed a significant overall effect of "protocol" ($F_{(2,34)} = 10.01$, $p = 0.0004$), no effect of "test session" ($F_{(3,102)} = 0.623$, $p = 0.602$) and a marginally significant interaction between "protocol" and "test session" ($F_{(6,102)} = 1.945$, $p = 0.081$). Post hoc analysis revealed that rats in the "standard" protocol showed significantly higher levels of prosocial choices as compared to the two other behavioral protocols, except for session 1, where it differed from the "no display of preference" ($p' = 0.023$), but not the "reward on both sides" protocols ($p' = 0.179$) (which in turn was not significantly different from the "no display of preference" protocol [$p' = 0.183$]; see Figure 2B). These results further support the finding that food-seeking behavior is necessary, but not sufficient, to drive prosocial choices in focal rats and that focal animals are sensitive to the reward received by recipients. In addition, in the first session, rats in the "no display of preference" group showed lower levels of prosocial choices than rats in the other two groups (although the difference relative to rats in the "reward on both sides" protocol did not reach significance), suggesting that at early stages of testing local enhancement or behavior copying (triggered by the food-seeking behavior of recipients) may have facilitated learning. This would, however, not be sufficient to sustain a preference for the prosocial side, as only rats in the "standard" protocol reliably and gradually acquired this preference.

We also asked whether prosocial choice was a goal directed or a habitual choice. To this end, we trained an independent set of animals in a similar manner as for the "standard" protocol, and, after 4 days of testing, we changed contingencies by providing the recipient with reward on both sides (instead of reward delivery only on the prosocial side). Importantly, reward to the focal rat remained unchanged. If prosocial choice were a habitual action, we would expect focal rats to keep their preference for the prosocial side despite the change in the contingency between the focals' choice and reward delivered to recipients. However, we observed a drop in the preference for the prosocial choice when recipients were suddenly rewarded on both sides (see Figure S2B).

The apparent sensitivity of focal rats to the delivery of reward to recipients, as seen by the lack of preference for the prosocial side when rewards to recipients were delivered on both sides of the maze, may have resulted from the fact that recipient rats could be less motivated to display food-seeking behavior as they would be rewarded on both arms and, despite their training, poked less on the "prosocial" side to which they were trained. To address this issue, we quantified the number of pokes on the prosocial side displayed by recipient rats before the focal made its decision in the first ("standard" condition) and third ("reward on both sides") experiments, and we found no difference (Figure 3A; Mann-Whitney U test, $U = 57.5$, $p' = 0.198$ for average prosocial pokes over sessions). Pokes on the opposite, "selfish," side were negligible in both experiments. Moreover, the number of pokes made by recipients was similar between prosocial and selfish trials (data not shown). Hence, recipients' food-seeking behavior, expressed as nose poking on the trained side, was similar across the two experimental conditions and across trial type. This is expected since in both protocols during individual training, recipient rats had to poke on average five times in order to gain access to food, and during testing nose poking by the recipient was necessary to activate the focal's nose ports and thus to progress within the trial. We also quantified the number of times the recipient rat investigated the focal rat during the display of food-seeking behavior (between the start of the trial and the focal's decision) and again found no difference between the two protocols (see Social Interactions below and Figure S4). This result further supports the similarity in the recipients' behavior before the decision was made, to the extent that we could quantify, across the two protocols.

Sensitivity to Reward Rate and Temporal Discounting

We noticed that in some trials of the "standard" condition, where the focal chose the selfish choice, recipients were reluctant to enter the arm, thus delaying the beginning of the next trial (which required both rats to be back in the center arm). Indeed, the time elapsed between the moment the focal opened the doors and the entry of both rats in the reward area was longer for selfish than prosocial trials in the "standard" and "no display of preference" protocols (Figure S3A). These correspond to the two experimental conditions in which reward was delivered to the recipient on one side only. The systematic delay in selfish trials could lead to fewer trials, and therefore fewer rewards, on those

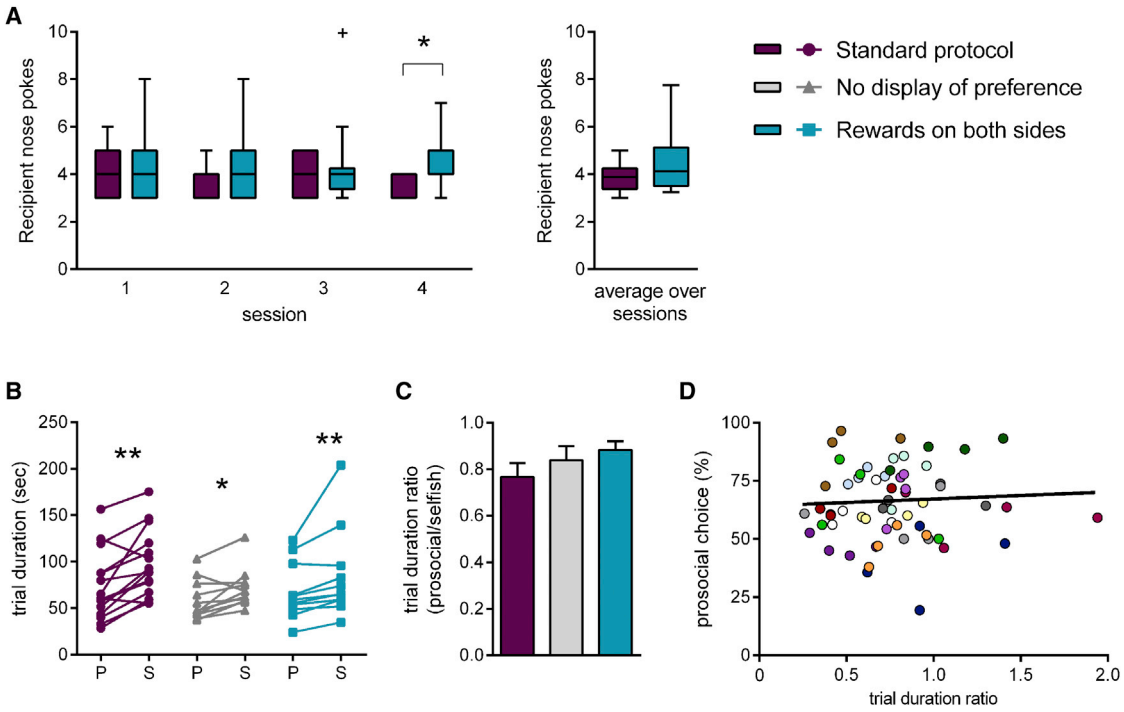

Figure 3. Recipients Display Similar Pre-decision Food-Seeking Behavior across Protocols and Focal's Reward Rate Does Not Predict Choice

(A) The number of nose pokes in the rewarded side displayed by recipients, before the focal made its choice, was similar in the "standard" and "reward on both sides" protocols during each test session (left) and averaged across sessions (right), except for the last session of prosocial testing, in which recipients of the "standard" protocol nose poked significantly less (Mann-Whitney test: $U = 81.5$, $p' = 0.959$ for session 1; $U = 59.5$, $p' = 0.237$ for session 2; $U = 74.5$, $p' = 0.683$ for session 3; $U = 31.0$, $p' = 0.024$ for session 4; $U = 57.5$, $p = 0.198$ for recipient pokes averaged over sessions).

(B) The median duration of selfish trials (S) was higher than that of prosocial trials (P) in all three protocols (paired-sample Wilcoxon signed-rank test within each experimental protocol: $Z = -3.010$, $p' = 0.008$ for the "standard" protocol; $Z = -2.311$, $p' = 0.021$ for the "no display of preference" protocol; $Z = -2.845$, $p' = 0.009$ for the "reward on both sides" protocol). See also Figure S3.

(C) When the ratio between the duration of prosocial and selfish trials was calculated for each individual animal, no differences between protocols were observed (one-way ANOVA: $F_{(2,34)} = 1.154$, $p = 0.327$).

(D) Scatter plot showing percentage of prosocial choice and prosocial/selfish trial duration ratio (for each rat, four data points are shown, one per session). Pearson's correlation between the two variables was not significant, confirming that trial duration (or reward rate) was not predictive of choice. Different animals are color coded.

In (A), box plots show median, first and third quartiles, and minimum and maximum values. Crosses represent outliers. Mean ± SEM are shown in (C). *$p' < 0.05$, **$p' \leq 0.005$. Corrected p values (p') after sequential Bonferroni correction for multiple comparisons are reported; four comparisons were performed in (A) and three in (B).

sessions where more "selfish" choices were made, raising the possibility that animals in the "standard" protocol chose the prosocial side more often in order to maximize their own reward [39]. We compared the number of trials across protocols for all sessions and found no differences in the total number of trials performed by rats in the three experimental protocols; hence, focal rats in the different protocols received a similar number of rewards (Figure S3B). Still, longer selfish trials imply (1) a higher reward rate for rats choosing more often the prosocial side and (2) a delay (relative to prosocial trials) in starting a new trial and hence the opportunity to get another reward. This could lead to temporal discounting (i.e., attribution of lower value to delayed reward) of food reward on the selfish side, which in turn could have influenced the choices of focal rats. Indeed, temporal discounting has been proposed as a crucial factor in social decision-making and cooperative behavior [40].

Therefore, we compared median trial duration of prosocial and selfish choice trials in all three conditions. Selfish trials were significantly longer than prosocial trials in all three experimental protocols (Wilcoxon signed-rank test within each protocol: $Z = -3.01$, $p' = 0.008$ for the "standard" protocol; $Z = -2.31$, $p' = 0.021$ for the "no display of preference"; and $Z = -2.85$, $p' = 0.009$ for the "reward on both sides" conditions) (Figure 3B). As variability in trial duration across interacting dyads could mask differences between experimental conditions, we computed the ratio of median trial duration between prosocial and selfish trials for each rat. Still, no difference was found across conditions (Figure 3C; one-way ANOVA: $F_{(2,34)} = 1.154$, $p = 0.327$). This finding indicates that the observed increase in reward rate on the prosocial side was not sufficient to drive a preference for that side, as in both the "no display of preference" and "reward on both sides" conditions no preference for the prosocial side was seen despite the increased reward rate associated with choosing that side. It was still possible that the difference observed between prosocial and selfish trial duration could explain the variance observed in the proportion of prosocial

choices displayed by focals in the ''standard'' condition (see Figure 2D). To examine this possibility, we performed a Pearson's correlation across the proportion of prosocial choice (displayed by each rat on each of the four test sessions) and the ratio between prosocial and selfish trial duration. We found no correlation between these two measures ($r = 0.030$, $p = 0.82$; Figure 3D). In addition, the relative difference in trial duration only emerged in session 3 (Figures S3C–S3F), whereas a preference for the prosocial side was apparent in the very first test session (see Figure 2B).

However, since rodents have been shown to perceive absolute time differences [41], it is possible that in our task the focal rats were not sensitive to the relative difference between prosocial and selfish trial duration (as calculated with the ratio), but rather were responding to absolute time differences which could differ between experimental protocols. To evaluate this possibility, we calculated the median difference in trial duration between trial types focusing on the two experimental protocols in which recipients were rewarded only in one side (i.e., ''standard'' and ''no display of preference'' protocols). We found that (1) absolute time difference in trial duration was not significantly different between experimental protocols over days (a repeated-measures ANOVA with ''experimental protocol'' as a between-subject factor and ''session'' as a within-subject factor revealed a significant effect of ''session'' [$F_{(3,78)} = 5.959$, $p = 0.0.001$], no significant effect of ''experimental protocol'' [$F_{(1,26)} = 1.205$, $p = 0.282$], and no significant interaction of testing session by experimental protocol [$F_{(3,78)} = 0.206$, $p = 0.892$]), (2) as observed with the ratio, absolute time differences emerged later than a preference for the prosocial choice, being only significant from the third testing session onward (data not shown), and that (3) there was no correlation between choice and absolute time difference ($r = -0.050$, $p = 0.703$ for the ''standard'' protocol; $r = -0.175$, $p = 0.225$ for the ''no display of preference'' protocol).

Taken as a whole, these findings make reward rate and temporal discounting as the main driving force of prosocial choice highly unlikely. The fact that time difference (absolute or relative) between prosocial and selfish trials emerged later than the preference for prosocial choice indicates that reward rate and temporal discounting are not sufficient to initiate prosocial choice. This is possibly due to the fact that rats were not food deprived and that in all experiments rewards were available to the focal rat as soon as it made its choice, such that the delay to receive the reward (from nose poking to pellet retrieval from the food magazine) was independent of the recipient's behavior and hence of trial type. However, we cannot exclude the possibility that these factors could play a role in the maintenance of choice in later testing sessions.

Social Interactions

In all experiments, rats had ample opportunity to interact, which could affect the decisions made by focal rats. Therefore, we examined how the interactions between focal and recipient rats might relate to the focals' choices. To this end, we quantified the number of times focal and recipient rats investigated each other, the number of times the focal unilaterally investigated the recipient, and vice versa. Given that the three forms of social investigation followed the same pattern (see Figures S4A–S4D for an analysis of each type of interaction), we used the sum of all three forms of interaction, henceforth referred to as social investigation. The amount of social investigation was similar in the ''standard'' and ''reward on both sides'' protocol, being higher than in the ''no display of preference'' protocol, as in the latter group rats could not interact before the focal made its decision (Figure 4A, left panel). In addition, for all three conditions, the number of interactions was highest on the first test session (Figure 4A, right panel).

One possibility is that social investigation is not homogenous across different stages of the trial, such as before and after the focal's decision, and differences across experimental protocol would only be apparent in a particular segment of the trial. Hence, we next divided the trials in three segments and analyzed the number of social investigation bouts in each of the following: (1) investigation bouts taking place before the focal made its choice (only possible in the ''standard'' and the ''reward on both sides'' conditions), (2) investigation bouts from the moment the focal made its decision until the recipient retrieved its food (or in selfish trials until the recipient entered the lateral unrewarded arm), and (3) investigation bouts from the moment the recipient retrieved its reward (or in selfish trials until the recipient entered the lateral unrewarded arm) until both rats were back to the central arm to initiate another trial. We found that the number of social investigation bouts was similar across protocols in the different trial segments, except for the increased number of social investigation in rats of the ''no display of preference'' condition during the time between the focals' decision and the recipients' retrieval of food. This is possibly because rats in this experimental group could not interact before the focal made its decision (one-way ANOVA: $F_{(1,24)} = 1.447$, $p = 0.241$ for ''prior to decision''; $F_{(2,33)} = 19.477$, $p < 0.0001$ for ''from decision to recipient's reward''; LSD post hoc: ''standard'' against ''no display of preference,'', $p' < 0.0001$; ''standard'' against ''reward on both sides,'', $p' = 0.068$; ''reward on both sides'' against ''no display of preference,'' $p' < 0.0001$; $F_{(2,33)} = 1.780$, $p = 0.184$ for ''from recipient's reward to trial end''; Figure 4B).

Finally, we assessed differences in social investigation between prosocial and selfish trials for all experimental protocols, as these could contribute to the decisions of focal rats. We found that prior to the focal rats' decision, the number of social investigation bouts was similar across trial type (Figure S4E), suggesting that social interactions before the focal rat made its decision were not related to prosocial choice. However, after the focal rat made its decision, rat dyads in the ''standard'' and ''no display of preference'' protocols displayed higher levels of social investigation in selfish trials relative to that observed in prosocial trials (paired-sample Wilcoxon signed-rank tests between prosocial and selfish trials for each independent protocol: $Z = -3.408$, $p' = 0.001$ for the ''standard'' protocol; $Z = -2.803$, $p' = 0.015$ for the ''no display of preference'' protocol; $Z = -1.156$, $p' = 0.248$ for the ''reward on both sides'' protocol; Figure 4C, left panel). We calculated the ratio between the amount of social investigation displayed in prosocial and selfish trials for each protocol and found that this ratio was highest in the ''standard'' protocol (one-way ANOVA: $F_{(2,33)} = 31.478$, $p < 0.0001$; LSD post hoc: ''standard'' against ''no display of preference'' protocol, $p' = 0.004$;

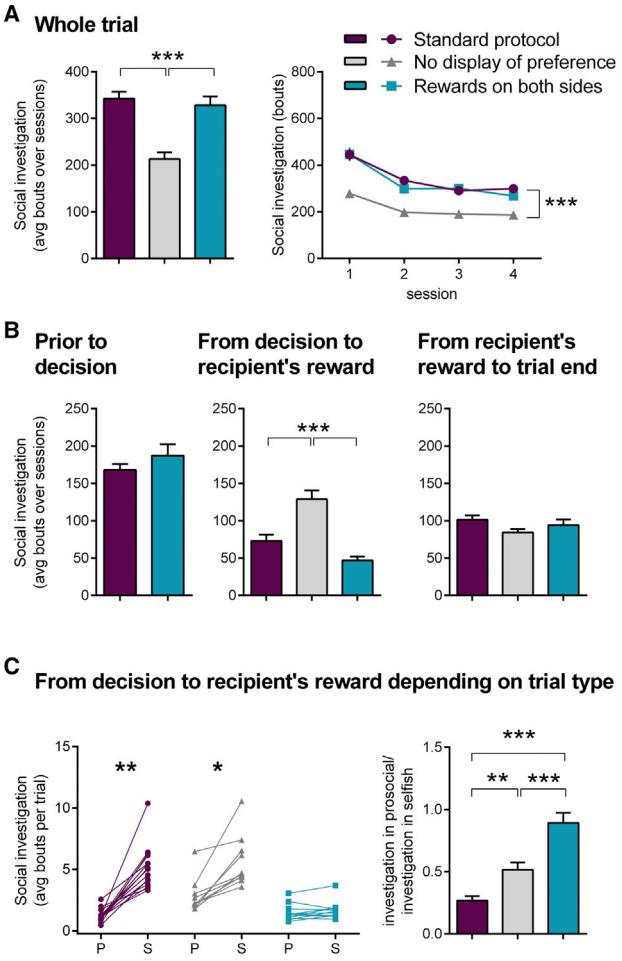

Figure 4. Social Investigation after the Focal Makes Its Decision Is Higher in Selfish Than Prosocial Trials

(A) The amount of social investigation between animals was lower in the "no display of preference" protocol compared to the "standard" and "reward on both sides" protocols, which did not differ between each other. The number of social investigation bouts decreased over sessions in a similar manner in all experimental protocols (repeated-measures ANOVA with "session" as a within-subject factor and "protocol" as a between-subjects factor confirmed differences between the protocols [$F_{(2,33)} = 17.73$, $p < 0.00001$]; further LSD post hoc confirmed lower levels of social interaction in all testing sessions compared to the "standard" [$p' = 0.00001$] and "reward on both sides" [$p' = 0.00009$] protocols) and a decrease of the amount of social investigation bouts over testing sessions ($F_{(3,99)} = 59.184$, $p < 0.00001$) that was similar in all experimental protocols ("session" × "protocol" $F_{(6,99)} = 2.036$, $p = 0.068$).

(B) No differences in the amount of social investigation (1) prior to the focal's decision, (2) from the moment of the decision until both animals were in the reward areas, and (3) from reward to trial end were observed between the protocols, except for the increased number of social investigation in rats of the "no display of preference" protocol in (2).

(C) Rat dyads from the "standard" and "no display of preference" protocols showed increased social investigations in selfish trials from the moment of decision (the focal pokes the nose port) until the recipient's reward delivery. This difference in the number of social investigation bouts was more pronounced in the "standard" protocol, as seen by the ratio between social investigation in prosocial and selfish trials. See also Figure S4.

Mean ± SEM are shown in all panels, except for (C), where individual values are plotted. *$p' < 0.05$, **$p' \leq 0.005$, ***$p' \leq 0.001$. Corrected p values (p') after sequential Bonferroni correction for multiple comparisons are reported; three comparisons were performed in each graph.

"standard" against "reward on both sides" protocol, $p' < 0.0001$, "reward on both sides" against "no display of preference" protocol, $p' = 0.0002$; Figure 4C, right panel). Since the difference in social investigation was observed in the two protocols in which the recipient received food only on one side, this result suggests that reward delivery influenced the way in which rats interact, which in turn may have influenced prosocial behavior. More specifically, the lack of reward delivery to recipient rats in selfish trials may have driven more social investigation by the dyad. The fact that a similar pattern is observed for all three forms of social investigation—mutual investigation, focal investigates recipient, and vice versa—suggests that when one rat investigates the other, the target of investigation tends to investigate back (see Figure S4G). Given the limitations in the accuracy of our behavioral analysis for the short investigation bouts that we measured (average bout duration: 0.97 ± 0.017 s), we could not determine who was driving the interaction, the focal or the recipient rat. Still, as it is the difference in reward received by the recipient that explains the differences in social investigation, it is possible that the observed increase in social investigation in selfish trials at the reward area may have been driven by recipient rats, which could be akin to begging or harassment displays observed in primates [1, 4, 22]. These interactions, together with the display of food-seeking behavior (nose poking), may have led to increased prosocial choices by focal rats in the "standard" condition.

Conclusions

We found that rats were prosocial in a food-foraging task, providing access to food to a cage-mate in the absence of added self-benefit or cost. This behavior was modulated by the display of food-seeking behavior expressed as poking a nose port on the door that gave access to the food-baited arm and by social interactions during the task.

By demonstrating that rats provide food to others in the absence of a direct or deferred benefit within the context of the task, we expand previous studies on prosocial behavior in rats reporting that these animals provide food to others in reciprocity-based tasks [8, 9] and that they relieve others from stress in the absence of self-benefit [17, 18]. Attempts to find evidence of provision of food to others under laboratory settings have provided conflicting evidence, possibly due to the fact that for animals to display this form of prosocial behavior they may have to overcome the drive to compete for food (even when subjects are not competing for food directly) [16, 42]. Rats have been shown to compete for food in a foraging task [43]; however, early descriptions of rats' natural behavior report that these animals are often found feeding in groups, showing tolerance for the presence of others at a food site even in conditions of limited food resources [36, 37].

Consistent with previous studies in primates [16, 21], we found the display of food-seeking behavior—in our task, poking in the nose port that gave access to the food-baited arm—to be crucial for prosocial choices by focal rats. Focal animals followed the recipient's nose-poking behavior to make their choices, this factor being necessary for the emergence of prosocial choice. To our knowledge, this is the first evidence of gaze (body)-following behavior in rats, which could be an

important component of social coordination. Moreover, prosocial behavior was modulated by reward delivery to the recipient rat. Therefore, in our task, displays of food-seeking behavior were not sufficient to drive prosocial behavior. In addition, we found that focal rats were sensitive to a change in the contingency between their action and reward to the recipient rats, suggesting that the focals' choices were goal directed. The specific mechanism and sensory cues by which displays of food-seeking behavior and sensitivity to reward to a conspecific modulate prosocial choice remain to be established. Our results raise the possibility that vicarious reward signals reinforced prosocial choice. Vicarious reward signals have been shown in the brain of human and non-human primates [44–46]. Furthermore, recent reports show that the observation of a conspecific eating food drives an initial dopamine increase in the nucleus accumbens of rats [38] and that social reward signals are mediated by oxytocin and serotonin in the nucleus accumbens of mice [47]. Interestingly, vicarious reward signals are modulated by oxytocin in monkeys [48]. In addition to inducing vicarious reward signals, food delivery may have driven prosocial choice by altering the behavior of recipient rats. Indeed, this was the case in our task, since in selfish trials rats took longer to enter the reward area and displayed more bouts of social investigation. Whether vicarious reward signals in the rat brain can drive prosocial behavior and how the behavior of the recipient leads to prosocial choice remain to be established.

We believe that using a classical type of decision-making task in combination with the vast tools available in rodents to record and manipulate brain activity will greatly impact the search of the neural mechanism underlying prosocial behavior.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, two tables, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.05.018.

AUTHOR CONTRIBUTIONS

C.M. and M.A.M. conceived and designed the changes made to the automated double T-maze previously developed by S.M.R. to study cooperative behavior using two-choice tasks in rats. C.M. and M.A.M. designed all experiments. C.M. established the behavioral protocols. C.M. and D.F.C. performed all experiments. D.F.C. performed the offline video scoring of social interactions. S.M.R. analyzed the data regarding nose pokes, parsed the data obtained from the automated mazes, and aligned it to the video data. C.M. performed all statistical analysis. All authors discussed the data, and M.A.M. and C.M. wrote the paper.

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REFERENCES

1. Burkart, J.M., Fehr, E., Efferson, C., and van Schaik, C.P. (2007). Other-regarding preferences in a non-human primate: common marmosets provision food altruistically. Proc. Natl. Acad. Sci. USA *104*, 19762–19766.
2. Daniel, W.J. (1942). Cooperative problem solving in rats. J. Comp. Psychol. *34*, 361–368.
3. Drea, C., and Carter, A. (2009). Cooperative problem solving in a social carnivore. Anim. Behav. *78*, 967–977.
4. Horner, V., Carter, J.D., Suchak, M., and de Waal, F.B. (2011). Spontaneous prosocial choice by chimpanzees. Proc. Natl. Acad. Sci. USA *108*, 13847–13851.
5. Massen, J.J., van den Berg, L.M., Spruijt, B.M., and Sterck, E.H. (2010). Generous leaders and selfish underdogs: pro-sociality in despotic macaques. PLoS ONE *5*, e9734.
6. Owens, D.D., and Owens, M.J. (1984). Helping behaviour in brown hyenas. Nature *308*, 843–845.
7. Plotnik, J.M., Lair, R., Suphachoksahakun, W., and de Waal, F.B. (2011). Elephants know when they need a helping trunk in a cooperative task. Proc. Natl. Acad. Sci. USA *108*, 5116–5121.
8. Rutte, C., and Taborsky, M. (2007). Generalized reciprocity in rats. PLoS Biol. *5*, e196.
9. Rutte, C., and Taborsky, M. (2008). The influence of social experience on cooperative behavior of rats (Rattus norvegicus): direct vs generalised reciprocity. Behav. Ecol. Sociobiol. *62*, 499–505.
10. Scheid, C., and Noë, R. (2010). The performance of rooks in a cooperative task depends on their temperament. Anim. Cogn. *13*, 545–553.
11. Schneeberger, K., Dietz, M., and Taborsky, M. (2012). Reciprocal cooperation between unrelated rats depends on cost to donor and benefit to recipient. BMC Evol. Biol. *12*, 41.
12. Seed, A.M., Clayton, N.S., and Emery, N.J. (2008). Cooperative problem solving in rooks (Corvus frugilegus). Proc. Biol. Sci. *275*, 1421–1429.
13. St-Pierre, A., Larose, K., and Dubois, F. (2009). Long-term social bonds promote cooperation in the iterated Prisoner's Dilemma. Proc. Biol. Sci. *276*, 4223–4228.
14. Suchak, M., and de Waal, F.B. (2012). Monkeys benefit from reciprocity without the cognitive burden. Proc. Natl. Acad. Sci. USA *109*, 15191–15196.
15. Taborsky, M. (2013). Social evolution: reciprocity there is. Curr. Biol. *23*, R486–R488.
16. Yamamoto, S., Humle, T., and Tanaka, M. (2009). Chimpanzees help each other upon request. PLoS ONE *4*, e7416.
17. Ben-Ami Bartal, I., Decety, J., and Mason, P. (2011). Empathy and pro-social behavior in rats. Science *334*, 1427–1430.
18. Ben-Ami Bartal, I., Rodgers, D.A., Bernardez Sarria, M.S., Decety, J., and Mason, P. (2014). Pro-social behavior in rats is modulated by social experience. eLife *3*, e01385.
19. Schwab, C., Swoboda, R., Kotrschal, K., and Bugnyar, T. (2012). Recipients affect prosocial and altruistic choices in jackdaws, Corvus monedula. PLoS ONE *7*, e34922.
20. Tan, J., and Hare, B. (2013). Bonobos share with strangers. PLoS ONE *8*, e51922.
21. Warneken, F., Hare, B., Melis, A.P., Hanus, D., and Tomasello, M. (2007). Spontaneous altruism by chimpanzees and young children. PLoS Biol. *5*, e184.
22. Brosnan, S.F., and De Waal, F.B. (2003). Monkeys reject unequal pay. Nature *425*, 297–299.

23. Di Lascio, F., Nyffeler, F., Bshary, R., and Bugnyar, T. (2013). Ravens (Corvus corax) are indifferent to the gains of conspecific recipients or human partners in experimental tasks. Anim. Cogn. *16*, 35–43.
24. Silberberg, A., Allouch, C., Sandfort, S., Kearns, D., Karpel, H., and Slotnick, B. (2014). Desire for social contact, not empathy, may explain "rescue" behavior in rats. Anim. Cogn. *17*, 609–618.
25. Skerry, A.E., Sheskin, M., and Santos, L.R. (2011). Capuchin monkeys are not prosocial in an instrumental helping task. Anim. Cogn. *14*, 647–654.
26. Balter, M. (2012). Animal cognition. 'Killjoys' challenge claims of clever animals. Science *335*, 1036–1037.
27. Heyes, C. (2012). Simple minds: a qualified defence of associative learning. Philos. Trans. R. Soc. Lond. B Biol. Sci. *367*, 2695–2703.
28. Hollis, K.L., and Nowbahari, E. (2013). A comparative analysis of precision rescue behaviour in sand-dwelling ants. Anim. Behav. *85*, 537–544.
29. Vasconcelos, M., Hollis, K., Nowbahari, E., and Kacelnik, A. (2012). Prosociality without empathy. Biol. Lett. *8*, 910–912.
30. Decety, J. (2011). The neuroevolution of empathy. Ann. N Y Acad. Sci. *1231*, 35–45.
31. Schuster, R., and Perelberg, A. (2004). Why cooperate? An economic perspective is not enough. Behav. Processes *66*, 261–277.
32. Viana, D.S., Gordo, I., Sucena, E., and Moita, M.A. (2010). Cognitive and motivational requirements for the emergence of cooperation in a rat social game. PLoS ONE *5*, e8483.
33. Colman, A.D., Liebold, K.E., and Boren, J.J. (1969). A method for studying altruism in monkeys. Psychol. Rec. *19*, 401–405.
34. Silk, J.B., Brosnan, S.F., Vonk, J., Henrich, J., Povinelli, D.J., Richardson, A.S., Lambeth, S.P., Mascaro, J., and Schapiro, S.J. (2005). Chimpanzees are indifferent to the welfare of unrelated group members. Nature *437*, 1357–1359.
35. Tsoory, M.M., Youdim, M.B., and Schuster, R. (2012). Social-cooperation differs from individual behavior in hypothalamic and striatal monoamine function: evidence from a laboratory rat model. Behav. Brain Res. *232*, 252–263.
36. Barnett, S.A. (1958). An analysis of social behavior in wild rats. Proc. R. Soc. Lond. *130*, 107–152.
37. Ewer, R.F. (1971). The biology and behavior of a free-living population of black rats (Rattus rattus). Anim. Behav. Monogr. *4*, 127–174.
38. Kashtelyan, V., Lichtenberg, N.T., Chen, M.L., Cheer, J.F., and Roesch, M.R. (2014). Observation of reward delivery to a conspecific modulates dopamine release in ventral striatum. Curr. Biol. *24*, 2564–2568.
39. Steiner, A.P., and Redish, A.D. (2014). Behavioral and neurophysiological correlates of regret in rat decision-making on a neuroeconomic task. Nat. Neurosci. *17*, 995–1002.
40. Stephens, D.W., McLinn, C.M., and Stevens, J.R. (2002). Discounting and reciprocity in an Iterated Prisoner's Dilemma. Science *298*, 2216–2218.
41. Gallistel, C.R., and Gibbon, J. (2000). Time, rate, and conditioning. Psychol. Rev. *107*, 289–344.
42. Brosnan, S.F., Silk, J.B., Henrich, J., Mareno, M.C., Lambeth, S.P., and Schapiro, S.J. (2009). Chimpanzees (Pan troglodytes) do not develop contingent reciprocity in an experimental task. Anim. Cogn. *12*, 587–597.
43. Hillman, K.L., and Bilkey, D.K. (2012). Neural encoding of competitive effort in the anterior cingulate cortex. Nat. Neurosci. *15*, 1290–1297.
44. Chang, S.W., Gariépy, J.F., and Platt, M.L. (2013). Neuronal reference frames for social decisions in primate frontal cortex. Nat. Neurosci. *16*, 243–250.
45. Chang, S.W., Winecoff, A.A., and Platt, M.L. (2011). Vicarious reinforcement in rhesus macaques (macaca mulatta). Front. Neurosci. *5*, 27.
46. Mobbs, D., Yu, R., Meyer, M., Passamonti, L., Seymour, B., Calder, A.J., Schweizer, S., Frith, C.D., and Dalgleish, T. (2009). A key role for similarity in vicarious reward. Science *324*, 900.
47. Dölen, G., Darvishzadeh, A., Huang, K.W., and Malenka, R.C. (2013). Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. Nature *501*, 179–184.
48. Chang, S.W., Barter, J.W., Ebitz, R.B., Watson, K.K., and Platt, M.L. (2012). Inhaled oxytocin amplifies both vicarious reinforcement and self reinforcement in rhesus macaques (Macaca mulatta). Proc. Natl. Acad. Sci. USA *109*, 959–964.